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Determining the Frequency of Antibiotic Sensitivity in Blood Infections in Ardabil province in 2010-2011

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ABSTRACT

Determination of the frequency of bacteria isolated from blood culture and the pattern of their antibiotic sensitivity is epidemiologically of great importance and can help choosing primary antimicrobial treatment. The aim of the current study was to determine the most common bacterial factors of blood infection and their antibiotic sensitivity in a one year period from October 2010 till the end of September 2011 in Imam Khumeini hospital of Ardebil. Data collected from total of 1469 patients susceptible to blood infection hospitalized in Imam Khumeini hospital and analyzed using SPSS (version 18) statistical software and investigated with descriptive statistics and frequency diagrams. Only values with $P \leq 0.05$ were considered as significant. The highest rate of bacteremia prevalence observed in emergency unit. Of 1469 blood sample, 91 (6.19%) were infected with bacteria. Infection prevalence reported higher in male (55.4%) than to female (44.6%). The most common bacteria isolated were, Negative-coagulase Staphylococcus (37.3%), Escherichia coli (22%), Staphylococcus aureus (11%) and Staphylococcus epidermidis (11%), respectively. The highest antibiotic sensitivity was associated with Gentamicin with the frequency rate of 42 (51.3%) and Ciprofloxacin with the frequency rate of 35 (42.7%). Gentamicin and Ciprofloxacin determined as two completely effective antibiotics on isolated strains. Regarding the increased prevalence of infection with Negative-coagulase Staphylococcus in the current study and other similar studies, the morbidity and mortality rate of the disease can be decreased using proper antibiotic treatments complying with the exact results of the disc diffusion test method.

1. Introduction

Circulatory system which is considered a sterile environment in normal conditions can be infected after the bacteria enters into the blood circulation which is called bacteremia (Remington et al., 1995). Bacteremia caused by different kinds of gram positive and negative bacteria and is diagnosed by blood culture experiments. Pathogens enter into the blood via digestive, uro-genital systems, skin and bones (Behrman et al., 2007). Since the cause of bacteremia in different parts of the world is different, so it is necessary to know the prevalence and epidemiological properties of the pathogenes (Archer et al., 2008). Bacteremia is seen in people with immunodeficiency system, who performed operation or under medical control, old population, patients that received invasive treatments or who use implant (Reynolds et al., 2004). Identifying of live organisms in the blood of patients is of

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diagnostic and prognostic importance (Weinstein et al., 2003). According to the studies, number of the people who lost their life because of the blood infection in last 20 years has been two-folded. One of the reasons is that the surgeons are treating cancers and implanting organs. In these kinds of operations, the used drugs and medications weaken immune system (Weinstein et al., 1983). Due to the immunodeficiency defensive mechanism and exposure to different invasive organisms, the spread of infection cannot be prevented, and 25-40% of patients with bacteremia suffering from meningitis (Forfar et al., 1984). Obtaining adequate knowledge on the frequency and antibiotic sensitivity of bacterial factors isolated from clinical samples all over the country, provide valuable information in choosing the best antibiotic to treat the patients. These studies should be persistently performed in all community, because it is proved that the type of the bacteria isolated in blood culture and also the antibiotic resistance varies by time (Weinstein et al., 1983). In a study in San Francisco (USA) on bacteria isolated in blood culture in 1996-1999, an increased in antibiotic resistance in most bacteria was reported (Huang et al., 2002). The aim of the current study was to investigate the frequency of different bacteremia factors and the condition of their disc diffusion test pattern in patients hospitalized in Imam Khumeini hospital of Ardebil during a one year.

2. Materials and Methods

This is a descriptive study relying on data available in Imam Khumeini hospital of Ardebil. Results of the all blood cultures for 1469 cases that were examined from October 2010 until September 2011, obtained from the laboratory records. Patients information including age, gender and hospitalization ward, and also information related to disc diffusion test and the results of culture recorded in check-list, regarding the computerized recordings and written reports of the laboratory, respectively. In the microbiology laboratory, after taking blood of the volunteer patients, the samples were maintained in incubator for 7-10 days in 37°C. After 24 hours, a sub-culture of the samples performed in mechanical environments and blood agar. If the result was negative the second sub culture was done after 72 hours and if the result was negative, another subculture has been performed after 10 days. In the case of turbidity in any stage, diffusion of the isolated colonies was made using gram stain method. Catalase test was performed in order to identify the gram positive coccus. In the case of being negative, sensitivity to Bacitracin disc and bacterial sensitivity in 60°C in 30 minutes and 6.5% NaCl resistance used to identify *Streptococcus pyogenes* and *Enterococcus*, respectively. If the result of the catalase test was positive, coagulase test used to identify coagulase-positive *Staphylococcus*.

However, SIM, TSI Agar, Simmons citrate agar and urea agar used to identify gram negative bacilli and then isolates were identified (Baron et al., 1994). Disk diffusion test performed on all positive cases using disk diffusion method (DDM) and Kirby and Bauer Method with recommendation of National Committee of Clinical Laboratory Standards (NCCLS) and the applied antibiotic disks made by Padtan Teb of Iran Co. contains of Amikacin (30µg), Gentamicin (10µg), Ceftriaxone (30µg), Vancomicin (30µg), Oxacillin (1µg), Cefotaxime (30µg), Piperacillin (100µg), Cefazolin (30µg), Imipeneme (10µg), Ciprofloxacin (5µg), Ceftizoxime (30µg), Cefixime (5µg), Ampicillin (10µg), Cephalothin (30µg), Cefalexin (30µg), Chloramphenicol (30µg), Trimethoprim (30µg).

First of all, a suspension of isolated colonies was made according to 1.5×10^8 CFU/ml. Immediately after that the samples approached the turbidity and it was extracted from bacterial suspension using sterile swap and was totally plated on Muller Hinton agar culture and after a few minutes, antibiotic disks were placed on a plate surface in proper distance using sterile swap and then investigated after 18-24 hours of incubation in 35-37°C. Inhibition zone enclosing every disk measured with a ruler up to 1 mm resolution and determined by standard table. The results compared with latest accessible standard tables to identify the antibiotic sensitivity of isolated bacteria (Falsafi et al., 2004).

Data analysis has been done via SPSS software (version 18) and also was investigated via frequency distribution tables and descriptive statistics ($P<0.05$ was regarded as meaningful).

3. Result

The average age and standard deviation of patients was 55.52±23, the youngest with one years

of age and the oldest with 95 years of age of whom 55.4% were male and 44.6% were female. 91(6.19%) of all of the patients were positive related to bacteria in the blood. Age group of bacteremia patients was 3-90 with average age and standard deviation of 60.48±20.28 of whom 50.5% were male and 49.5 were female.

The oldest average age included *Pseudomonas* sp. bacteria (82 years of age) with standard deviation of 3 and *Escherichia coli* (65.9 years of age) with standard deviation of 3.63 and the youngest average age was related to *Enterobacter* sp. (49.75 years of age) with standard deviation of 10. Distribution of age variables reported normal using K-S test. There was not any significant difference between the qualitative variable of gender and the bacteremia infection (P>0/05) and also no significant difference observed in isolated bacteria of the two genders. Regarding the frequency of the isolation of different bacteria of blood culture of the patients in Table 1, the bacteria are Negative-coagulase *Staphylococcus*, *Escherichia coli*, *Staphylococcu saureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticous*, *Enterobacter* sp., *Klebsiella* sp., *Pseudomonas* sp., *Acinetobacter sp.*, *Proteus mirabilis*, *Citrobacter sp.* and *Streptococcus* viridians, respectively. Four prevalent bacteria in blood culture were negative-coagulase *Staphylococcus*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* with the frequency of 37.3%, 22%, 11% and 11% respectively.

In the current study, the highest frequency of the organism was associated with negative-coagulase *Staphylococcus* 34(37.3%) and the lowest frequency was associated with *Acinetobacter sp.*, *Proteus mirabilis*, *Citrobacter* sp. and *Streptococcus* viridans 1(1.1%).

The frequency rate of bacteria in 11 wards of the understudy hospital was different, for example, the frequency rate of bacteria was highest in emergency unit with 68 cases (74/7%).

Results of the experiment determining the antibiotic sensitivity of the bacteria prevalent in blood culture of the patients is shown in Table 3.

Table 1: Relative frequency of bacteria isolated from bacteremia affected patients.

Bacteria isolated from blood culture	Number	(%)
Coagulase-negative *Staphylococcus*	34	37.3
E.coli	20	22
S.aureus	10	11
S.epidermidis	10	11
S.saprophyticous	4	4.4
Enterobacter sp.	4	4.4
Klebsiella sp.	3	3.3
Pseudomonas sp.	2	2.2
Acinetobacter sp.	1	1.1
Proteus mirabilis	1	1.1
Citrobacter sp.	1	1.1
Streptococcus viridans	1	1.1
Total	91	6.19

Table 2: The frequency distribution of bacteria isolated from patients hospitalized in different wards of the understudy hospital.

ward	Frequency	(%)
Emergency	68	74.7
ICU	9	9.9
Women internal unit	4	4.4
Men internal unit	3	3.3
Women Infection	3	3.3
Men Infection unit	3	3.3
Angiography unit	1	1.1
Women surgery	-	-
Men surgery	-	-
Cardiography	-	-
CCU	-	-

Table 3: Sensitivity of the bacteria prevalent in blood culture to the understudy antibiotics.

Antibiotic	*E.coli*		*S.aureus*		Negative-coagulase		*S.epidermidis*	
	frequency	(%)	Frequency	(%)	frequency	(%)	frequency	(%)
Amicacin	12	14.6	0	0	0	0	0	0
Gentamicin	10	12.2	4	4.9	19	23.2	9	11
Ceftriaxone	5	6.1	5	6.1	8	9.8	3	3.7
Vancomycin	0	0	5	6.1	11	13.4	3	3.7
Oxacillin	0	0	1	1.2	0	0	1	1.2
Cefotaxime	4	4.9	1	1.2	2	2.4	0	0
Piperacillin	0	0	0	0	1	1.2	0	0
Cefazolin	1	1.2	2	2.4	3	3.7	1	1.2
Ciprofloxacin	6	7.3	6	7.3	18	22	5	6.1
Ceftizoxime	0	0	1	1.2	0	0	0	0
Cefixime	5	6.1	0	0	1	1.2	0	0
Trimethoprim	5	6.1	6	7.3	12	14.6	7	8.5
Ampicillin	1	1.2	1	1.2	3	3.7	0	0
Imipeneme	0	0	0	0	1	1.2	1	1.2
Cephalothin	0	0	1	1.2	0	0	0	0
Chloramphenicol	O	0	0	0	2	2.4	0	0
Cefalexin	3	3.7	4	4.9	14	17.1	7	8.5

Sensitivity rate varies in different bacteria but the highest antibiotic sensitivity in four isolated bacteria was associated with Gentamicin and Ciprofloxacin with the frequency of 42 (51.3%) and 35 (42.7%), respectively. Sensitivity to Gentamicin in prevalent *Escherichia coli* strains, *Staphylococcus aureus*, *Staphylococcus epidermidis* and negative coagulase *Staphylococcus* was 10 (12.2%), 4 (4.9%), 9 (11%), 19 (23.2%) and their sensitivity to Ciprofloxacin was 6 (7.3%), 6 (7.3%), 5 (6.1%) and 18 (22%), respectively.

4. Discussion

Many microbes can cause blood infection in which bacteria are among the most important factors. Because of the increased use of antibiotics which are not of bacteria causes (the viral ones), most bacteria developed resistance to different antibiotics that made it difficult to treat blood infection. Bacteremia-related mortality and also the increased antibiotic resistance of bacteria indicate performing continuous studies in this field. Thus, all of the results about blood culture of patients susceptible to Bacteremia is important epidemiologically. However, the isolation of bacteria from blood culture may not be of bacterial causes but may be due to an infection. So, the main criterion for choosing the samples of the study was making positive at least two of three blood samples (every sample with two hours interval) taken from patients hospitalized in different wards of the hospitals. If just one sample out of three was positive regarding the growth of negative-coagulase *Staphylococcus*, the sample excluded as an infection.

In the current study, the prevalence rate of bacteremia was (6.19%) and the most frequent isolated bacteria was negative-coagulase

Staphylococcus (37.3%) that is similar to the results of the studies of (Nemati et al., 1386) in Bandar Abbas. Prevalent bacterial found in blood culture in the current study reported in many other studies performed in Iran and abroad. In the current study, the most prevalent bacteria caused bacteremia were negative-coagulase *Staphylococcus* (37.3%), *Escherichia coli* (22%), *Staphylococcus aureus* (11%), *Staphylococcus epidermidis* (11%). In a study in 1990 on blood culture of 707 patients, the most prevalent bacteria reported as *Staphylococcus aureus*, *Escherichia coli*, Negative-coagulase *Staphylococcus*, *Klebsiella* sp. and *Enterobacter* sp. (Weinstein et al., 1997). In a study in Hamedan, *Staphylococcus epidermidis* was shown to be the most prevalent bacteria in 104 positive blood cultures (7.7%) (Yosefi et al., 1999). In the study of Hajizadeh and Daneshjoo in Imam Khumeini hospital, the most prevalent microorganisms isolated from 120 blood cultures samples was negative-coagulase *Staphylococcus* (25%) (Hajizadeh et al., 2003). In a study by Douglas, the isolated bacteria were *Staphylococcus aureus*, *Escherichia coli*, Negative-coagulase *Staphylococcus*, *Pseudomonas* sp. (Douglas et al., 2004), that the prevalence of *E.coli* complies with our study. A study performed in USA in 2002, reported the most prevalent bacteria isolated in blood culture including Negative-coagulase *staphylococcus* (42%), *Staphylococcus aureus* (16.5%), *Enterococcous fecalis* (8.3%), *E.coli* (7.2%), *Klebsiella pneumonia* (3.6%) and *Enterococcous fasioum* (3.5%) (Karlowsky et al., 2004).

In a retrospective study performed by Kara in the Turkey, from 8942 blood culture samples, 1000 (11.18%) were positive and the frequency of bacteria were negative-coagulase *Staphylococcus* (50.4%), *S.aureus* (8.1%), *Pseudomonas sp.* (5.3%), *Klebsiella sp.* (5%), respectively (Kara et al., 2004). In the study by Gray in England indicated that the most prevalent bacteria were gram positive (66.2%) and gram negative (31.3%) including Negative-coagulase *Staphylococcus*, *Staphylococcus aureus* and *Enterococcus*, respectively (Gray et al., 2004). In another study on 2248 isolation in pediatric center of Tehran from January 1996 till December 2000, the most prevalent bacteria were coagulase-negative *Staphylococcus* (48.4%), *S.aureus* (16.7%), *Klebsiella* sp. (8.5%), *E.coli* and *Pseudomonas sp.*, respectively (Mamishi et al., 2005). In the study by Blomberg in Tanzania, 13.9% of blood cultures were positive and the isolated bacteria were *Klebsiella* sp., *Salmonella* sp., *E.coli*, *Enterococcus* sp. and *S.aureus*, respectively (Blomberg et al., 2007). It is obvious that the frequency of isolation of any bacteria significantly varies in different studies, but the organisms are the same. Determining the most influential antibiotic on most bacteria isolated from blood culture can be useful in choosing the best medication for primary and emergency treatments in patients susceptible to bacteremia. In the current study, the highest sensitivity of the most prevalent bacteria was associated to Gentamicin and Ciprofloxacin. Another study also indicates the highest sensitivity of bacteria to Ciprofloxacin. In a study in Iran, Ciprofloxacin determined as the best medication for bacteremia related to gram negative bacteria (Yosefi et al., 1999). In another study using MIC (E-test) method on *E.coli*, the sensitivity to Gentamicin (100%) and Ciprofloxacin (100%) was reported (Leegaard et al., 2001). While in the current study, the sensitivity of *E.coli* to the above-mentioned antibiotics were (12.2%) and (7.3%), respectively, in which the difference in the frequency may be due to the using of different disc diffusion agar method, relative increase in resistance to antibiotics in understudy populations compared to other countries, difference in the quality of the antibiotic discs used in two studies (Martin et al., 1989).

However, since last decade, highest percent of bacteremia relates to the coagulase-negative *Staphylococcus* compared to *S.aureus*, and in some studies the three-folded rate was also reported (Katz et al., 2008). Regarding the above-mentioned cases and the highest prevalence of *Staphylococcous* species in understudy hospital, it is required to have more inspection and control on health condition of medical centers. Considering the highest rate of sensitivity of prevalent strains of bacteremia factors to Gentamicin and Ciprofloxacin, it seems that these antibiotics taken into account for the primary treatment of bacteremia. Particularly if it is likely that any gram negative *Bacillus* is present.

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